CLAIMS

1. A method of producing a binding moiety comprising
modifying an extracellular cytokine binding domain consisting of a first FnIII-like domain and a second FnIII-like domain such that at least one property of the cytokine binding domain is altered,
to produce a binding moiety.

2. The method according to claim 1, wherein the first and second FnIII-like domains are derived from the extracellular cytokine binding domain from a single source.

3. The method according to claim 1, wherein the first and second FnIII-like domains are derived from the extracellular cytokine binding domains from separate sources.

4. The method according to any preceding claim, wherein the first and/or second FnIII-like domain(s) is/are derived from the extracellular domain of a receptor selected from IL-2 receptor, IL-3 receptor, IL-4 receptor, IL-5 receptor, IL-6 receptor, IL-7 receptor, IL-9 receptor, IL-11 receptor, IL-12 receptor, IL-13 receptor, IL-15 receptor and IL-21 receptor, G-CSF receptor, GM-CSF receptor, LIF receptor, oncostatin M receptor, cardiotrophin CT-1 receptor, ciliary neutrotrophic factor (CNTF) receptor, prolactin receptor, leptin receptor, erythropoietin receptor, growth hormone receptor, cytokine receptor-like factor 1, class 1 cytokine receptor, thymic stromal lymphopoietin protein receptor or gp130.

5. The method according to any preceding claim, wherein at least one loop of the cytokine binding domain is modified.

6. The method according to claim 5, wherein the loops are defined by loops L1 to L7 as indicated in Figure 5.

7. The method according to claim 5 or claim 6, wherein the size and/or area of the loop is modified as compared with the corresponding loop in the unmodified cytokine binding domain.

8. The method according to claim 7, wherein the size of the loop is increased or reduced by at least two amino acid residues.

9. The method according to claim 8, wherein the size of the loop is increased by at least 10 amino acid residues

10. The method according to claim 8 or claim 9, wherein the size of the loop is increased by up to 20 amino acid residues.

11. The method according to any preceding claim, wherein the hinge region between the two FnIII-like domains is modified.

12. The method according to any preceding claim, wherein the binding interface of the FnIII-like domains of the cytokine binding domain is modified.

13. The method according to any preceding claim, wherein one or more intra-domain disulphide-bond forming cysteine residues in the cytokine binding domain are modified.

14. The method according to any preceding claim, wherein the solubility of the binding moiety is improved.

15. The method according to claim 14, wherein the solubility of the binding moiety is improved by removing and/or replacing disulphide-bond forming cysteine residues within in the cytokine binding domain.

16. The method according to any one of claims 13 to 15, wherein disulphide-bond forming cysteine residues are replaced by a different residue.

17. The method according to claim 16, wherein disulphide-bond forming cysteine residues are replaced by alanine or serine.

18. The method according to any preceding claim, wherein the stability of the cytokine binding domain is improved.

19. The method according to any preceding claim, wherein the affinity of the modified cytokine binding domain for at least one natural ligand of the unmodified cytokine binding domain is reduced or abolished.

20. The method according to any preceding claim, wherein the binding specificity of the modified cytokine binding domain is different to that of the unmodified cytokine binding domain.

21. The method according to claim 20, wherein the unmodified cytokine binding domain is derived from the extracellular domain of a first receptor having specificity for a first ligand, and the modification comprises replacing one or more loops of the unmodified cytokine binding domain with the corresponding loops of a second receptor having specificity for a second ligand such that the modified cytokine binding domain has specificity for the second ligand.

22. The method according to claim 21, wherein the first receptor is IL6 receptor and the second receptor is prolactin receptor or LIF receptor.

23. The method according to claim 22, wherein the first receptor is IL6 receptor and the second receptor is oncostatin M receptor.

24. The method according to any preceding claim, further comprising linking the modified binding moiety to one or more molecules.

25. The method according to claim 24, wherein the modified binding moiety is linked to one or more molecules via a genetic or chemical linker.

26. The method according to claim 24 or claim 25, wherein the modified binding moiety is linked to one or more molecules via a covalent or non-covalent linkage.

27. The method according to any one of claims 24 to 26, wherein the modified binding moiety is linked to a diagnostic reagent or a therapeutic agent.

28. The method according to claim 27, wherein the diagnostic reagent is a detectable label.

29. The method according to claim 27, wherein the therapeutic agent is cytotoxic.

30. The method according to claim 27, wherein the therapeutic agent is immunomodulatory.

31. A modified binding moiety produced by any one of the methods according to claims 1 to 30.

32. A binding moiety comprising an extracellular cytokine binding domain consisting of a first FnIII-like domain and a second FnIII-like domain, wherein the cytokine binding domain comprises a modification which alters at least one property of the cytokine binding domain.

33. The binding moiety according to claim 32, wherein the first and second FnIII-like domains are derived from the extracellular cytokine binding domain from a single source.

34. The binding moiety according to claim 33, wherein the first and second FnIII-like domains are derived from the extracellular cytokine binding domains from separate sources.

35. The binding moiety according to any one of claims 32 to 34, wherein the first and/or second FnIII-like domain(s) is/are derived from the extracellular domain of a receptor selected from IL-2 receptor, IL-3 receptor, IL-4 receptor, IL-5 receptor, IL-6 receptor, IL-7 receptor, IL-9 receptor, IL-11 receptor, IL-12 receptor, IL-13 receptor, IL-15 receptor and IL-21 receptor, G-CSF receptor, GM-CSF receptor, LIF receptor, oncostatin M receptor, cardiotrophin CT-1 receptor, ciliary neutrotrophic factor (CNTF) receptor, prolactin receptor, leptin receptor, erythropoietin receptor, growth hormone receptor, cytokine receptor-like factor 1, class 1 cytokine receptor, thymic stromal lymphopoietin protein receptor or gp130.

36. The binding moiety according to any one of claims 32 to 35, wherein a loop of the cytokine binding domain is modified.

37. The binding moiety according to claim 36, wherein the loops are defined by loops L1 to L7 as indicated in Figure 5.

38. The binding moiety according to claim 36 or claim 37, wherein the size and/or area of the loop is modified as compared with the corresponding loop in the unmodified cytokine binding domain.

39. The binding moiety according to claim 38, wherein the size of the loop is increased or reduced by at least two amino acid residues.

40. The binding moiety according to claim 39, wherein the size of the loop is increased by at least 10 amino acid residues.

41. The binding moiety according to claim 39 or claim 40, wherein the size of the loop is increased by up to 20 amino acid residues.

42. The binding moiety according to any one of claims 32 to 41, wherein the hinge region between the FnIII-like domains is modified.

43. The binding moiety according to any one of claims 32 to 42, wherein the binding interface of the FnIII-like domains is modified.

44. The binding moiety according to any one of claims 32 to 43, wherein one or more of intra-domain disulphide-bond forming cysteine residues in the cytokine binding domain is modified

45. The binding moiety according to any one of claims 32 to 44, wherein the solubility of modified binding moiety is improved.

46. The binding moiety according to claim 45, wherein the solubility of the binding moiety is improved by removing and/or replacing disulphide-bond forming cysteine residues within the cytokine binding domain.

47. The binding moiety according to any one of claims 44 to 46, wherein disulphide-bond forming cysteine residues are replaced by a different residue.

48. The binding moiety according to claim 47, wherein disulphide-bond forming cysteine residues are replaced with alanine or serine.

49. The binding moiety according to any one of claims 32 to 48, wherein the stability of the cytokine binding domain is improved.

50. The binding moiety according to any one of claims 32 to 47, wherein the affinity of the modified cytokine binding domain for at least one natural ligand of the unmodified cytokine binding domain is reduced or abolished

51. The binding moiety according to any one of claims 32 to 50, wherein the binding specificity of the modified cytokine binding domain is different to that of the unmodified cytokine binding domain.

52. The binding moiety according to claim 51, wherein the unmodified cytokine binding domain is derived from the extracellular domain of a first receptor having specificity for a first ligand, one or more loops of the unmodified cytokine binding domain have been replaced with the corresponding loops of a second receptor having specificity for a second ligand, and the modified cytokine binding domain has specificity for the second ligand.

53. The binding moiety according to claim 52, wherein the first receptor is IL-6 receptor and the second receptor is prolactin receptor or LIF receptor.

54. The binding moiety according to claim 52, wherein the first receptor is IL-6 receptor and the second receptor is oncostatin M receptor.

55. The binding moiety according to any one of claims 31 to 54 linked to one or more molecules.

56. The binding moiety according to claim 55, linked to one or more molecules via a genetic or chemical linker.

57. The binding moiety according to 55 or claim 56, linked to one or more molecules via a covalent or non-covalent linkage.

58. The binding moiety according to any one of claims 55 to 57, linked to a diagnostic reagent or a therapeutic agent.

59. The binding moiety according to claim 58, wherein the diagnostic reagent is a detectable label.

60. The binding moiety according to claim 58, wherein the therapeutic agent is cytotoxic.

61. The binding moiety according to claim 58, wherein the therapeutic agent is immunomodulatory.

62. A multivalent or multispecific reagent comprising two or more binding moieties according to any one of claims 31 to 61.

63. The binding moiety, multivalent reagent or multispecific reagent according to any one of claims 31 to 61, immobilised on a solid support or coupled to a biosensor surface.

64. A polynucleotide encoding a binding moiety, multivalent reagent or multispecific reagent according to one of claims 31 to 62.

65. A vector comprising a polynucleotide according to claim 64.

66. A host cell comprising a vector according to claim 65.

67. A pharmaceutical composition comprising a binding moiety, multivalent reagent or multispecific reagent according to any one of claims 31 to 62 and a pharmaceutically acceptable carrier or diluent.

68. A method of treating a pathological condition in a subject, which method comprises administering to the subject a binding moiety, multivalent reagent or multispecific reagent according to any one of claims 31 to 62.

69. A method of selecting a binding moiety with an affinity for a target molecule which comprises

(i) providing a plurality of polynucleotides encoding binding moieties comprising a cytokine binding domain, which polynucleotides comprise one or more modifications in the cytokine biding domain;

(ii) expressing the binding moieties encoded by the polynucleotides; and

(iii) selecting one or more binding moieties having an affinity for the target molecule.

70. The method according to claim 69, wherein the modification(s) is/are in the loop(s) of the cytokine binding domain.

71. The method according to claim 69 or claim 70, wherein the plurality of nucleotides have been subjected to mutagenesis.

72. The method according to claim 71, wherein the mutagenesis is site-directed mutagenesis.

73. The method according to claim 72, wherein the mutagenesis is random mutagenesis.

74. A method according to any one of claims 69 to 72, wherein the target molecule is a cytokine receptor ligand.

75. A polynucleotide library comprising a plurality of polynucleotides encoding binding moieties comprising a cytokine binding domain, which polynucleotides comprise one or more modifications in the cytokine biding domain.

76. A nucleic acid sequence encoding:
a) a first scaffold sequence encoding a cytokine binding domain; and
b) a second sequence encoding a peptide and inserted at a site located in a region of said first scaffold sequence, said peptide being displayed as a loop.

77. The nucleic acid sequence according to claim 76, wherein the second sequence substantially replaces the region of the first scaffold sequence encoding the loop.

78. The nucleic acid sequence according to claim 76 or claim 77, wherein the cytokine binding domain is derived from the extracellular domain of a receptor selected from IL-2 receptor, IL-3 receptor, IL-4 receptor, IL-5 receptor, IL-6 receptor, IL-7 receptor, IL-9 receptor, IL-11 receptor, IL-12 receptor, IL-13 receptor, IL-15 receptor and IL-21 receptor, G-CSF receptor, GM-CSF receptor, LIF receptor, oncostatin M receptor, cardiotrophin CT-1 receptor, ciliary neutrotrophic factor (CNTF) receptor, prolactin receptor, leptin receptor, erythropoietin receptor, growth hormone receptor, cytokine receptor-like factor 1, class 1 cytokine receptor, thymic stromal lymphopoietin protein receptor or gp130.

79. The nucleic acid sequence according to any one of claims 76 to 78, comprising a plurality of second sequences inserted into a plurality of sites.

80. The nucleic acid sequence according to any one of claims 76 to 79, wherein one or more of the peptides are derived from a different cytokine binding region to that of the scaffold sequence.

81. An expression vector comprising a nucleic acid sequence according to any one of claims 76 to 80.

82. A cytokine binding domain display library comprising a plurality of expression vectors according to claim 81.

83. An expression vector comprising:

a) a first nucleic acid sequence encoding a cytokine binding domain;

b) an insertion site in a region between the ends of the first nucleic acid sequence, the insertion site comprising a nucleotide sequence which is cleaved by a restriction endonuclease and which allows a second nucleic acid sequence encoding an amino acid sequence to be inserted into the first nucleic acid to encode a modified cytokine binding domain; and

c) a regulatory control sequence operably linked to said first nucleic acid sequence which directs expression of the first nucleic acid sequence.

84. An expression vector comprising:

a) a first nucleic acid sequence encoding a cytokine binding domain, said sequence comprising a deletion in a region between the ends of the first nucleic acid sequence;

b) an insertion site in place of the deleted sequence which site allows a second nucleic acid sequence encoding an amino acid sequence to be inserted into the first nucleic acid to encode a modified cytokine binding domain.

c) a regulatory control sequence operably linked to said first nucleic acid sequence which directs expression of the first nucleic acid sequence.

85. The expression vector according to claim 83 or claim 84, wherein the region in which the insertion site or deletion is present encodes a loop.

86. A polypeptide encoded by the nucleic acid sequence of any one of claims 76 to 80.

87. A protein multimer comprising at least two polypeptides according to claim 86.

88. A method of identifying a modified cytokine binding domain which binds to a target molecule of interest, which method comprises:

(i) providing a cytokine binding domain display library according to claim 82;

(ii) expressing the polypeptides encoded by the polynucleotides; and

(iii) selecting one or more polypeptides that bind to the target molecule.